INTRODUCTION

Fructooligosaccharides (FOS) are oligosaccharides of fructose containing a single glucose moiety; they are produced by the action of fructosyl transferase (FTase,) from many plants and microorganisms. The FOS formed contains fructosyl units bounded at the β-2, 1 position of sucrose; they are mainly composed by 1-kestose, nystose and 1-β- fructofuranosyl nystose (Sangeetha *et al.* 2005). Among FOS, the ones with low polymeric grade show better therapeutic properties than those with a high polymeric degree. They are about 0.4 and 0.6 times as sweet as sucrose and have been used in the pharmaceutical industry as a functional sweetener (Biedrzycka *et al.* 2004). FOS present properties such as low caloric values, non-cariogenic properties, decrease levels of phospholipids, triglycerides and cholesterol, help gut absorption of calcium and magnesium, are useful for diabetic products and are used as prebiotics to stimulate the bifidobacteria growth in the human colon (Modler *et al.* 1994). FOS are industrially produced from sucrose by microbial enzymes with transfructosylating activity. Most of these enzymes have been found in fungi such as *Aspergillus* sp., *Aureobasidium* sp., *Arthrobacter* sp. and *Fusarium* (Sangeetha *et al.* (2005).

Fructoologosaccharides are prebiotic substances found in several vegetables or natural foods. FOS are found in small amounts in vegetables such as onion, garlic, Jerusalem artichokes, asparagus, bananas, rye, wheat and tomatoes, They are calorie-free and non cariogenic sweeteners, stimulate the growth of *Bifidobacteria*, (Modler *et al.* 1994) and have been claimed to contribute towards the prevention of colon cancer and to reduce cholesterol, phospholipids and triglyceride levels in serum.

Fructooligosaccharides (FOS) also sometimes called oligofructose or oligofructan is a class of oligosaccharides used as an artificial or alternative sweetener. (Stamp *et al.* 1990) FOS exhibits sweetness levels between 30 and 50 percent of sugar in commercially-prepared syrups. Its use emerged in the 1980s in response to consumer demand for healthier and calorie-reduced foods. The term oligosaccharide refers to a short chain of sugar molecules (in the case of FOS, fructose molecules). *Oligo* means few, and *saccharide* means sugar.

Chemistry

Two different classes of fructooligosaccharide (FOS) mixtures are produced commercially, based on inulin degradation or transfructosylation processes. FOS can be produced by degradation of inulin, or polyfructose, a polymer of D-fructose

residues linked by β(2-1) bonds with a terminal α(1-2) linked D-glucose. The degree of polymerization of inulin ranges from 10 to 60. Inulin can be degraded enzymatically or chemically to a mixture of oligosaccharides with the general structure Glu-$(Fru)_n$ (GF_n) and Fru_m, (Fm), with n,m ranging from 1 to 7. This process also occurs to some extent in nature, and these oligosaccharides can be found in a large number of plants, especially in Jerusalem artichoke and chicory. This type of FOS is mainly marketed commercially by Orafti Ltd., Tienen Belgium, which markets the product as Oligofructose (or Raftilose). The company also markets inulin as oligofructose. Other producers include the Dutch company Cosun (which markets the product as Frutafit or Frutalose) and others. The main components of this class are kestose (GF2), nystose (GF3), fructosylnystose (GF4), bifurcose (GF3), inulobiose (F2), inulotriose (F3), and inulotetraose (F4).

The second class of FOS is prepared by the transfructosylation action of a β-fructosidase of *Aspergillus niger* on saccharose. The resulting mixture has the general formula of GF_n with n ranging from 1 to 5. Contrary to the inulin derived FOS, the binding is not only β(1-2), but other linkages do occur, though in limited numbers. This class is mainly produced in Japan by Meiji Seika Kaisha.

Because of the configuration of their osidic bonds fructooligosaccharides resist hydrolysis by salivary and intestinal digestive enzymes. In the colon they are fermented by anaerobic bacteria. In other words, they have a lower caloric value, whilst contributing to the dietary fiber fraction of the diet. Fructooligosaccharides are more soluble than inulins and are therefore sometimes used as an additive to yoghurt and other (dairy) products. Fructooligosaccharides are used specially in combination with high-intensity artificial sweeteners, whose sweetness profile and aftertaste it improves. FOS is extracted from fruits and vegetables like bananas, onions, chicory root, garlic, asparagus, barley, wheat, jícama, tomatoes, and leeks. The Jerusalem artichoke and its relative, yacón have been found to have the highest concentrations of FOS of cultured plants.

Health Benefits

FOS has been a popular dietary supplement in Japan for many years, even before 1990, when the Japanese government installed a "Functionalized Food Study Committee" of 22 experts to start to regulate "special nutrition foods or functional foods" that contain the categories of fortified foods (e.g., vitamin-fortified wheat

flour), and is now becoming increasingly popular in Western cultures for its prebiotic effects. FOS serves as a substrate for microflora in the large intestine, increasing the overall gastrointestinal tract (GI Tract) health. It has also been touted as a supplement for preventing yeast infections.

Several studies have found that FOS and inulin promote calcium absorption in both the animal and human gut. The intestinal microflora in the lower gut can ferment FOS, which results in a reduced pH. Calcium is more soluble in acid, and, therefore, more of it comes out of food and is available to move from the gut into the bloodstream (Van den Heuvel *et al.* 1999).

FOS can be considered a small dietary fiber with (like all fibers) low caloric value. The fermentation of FOS results in the production of gases and acids. The latter provide some energy to the body (Roberfroid *et al.* 1993).

In response to an increasing demand from consumer for healtheir and calorie controlled food a number of alternative sweeteners such as palatinose and various oligosaccharides including isomaltooligosachrides, soybeanooligosacchrides and fructooligosacchrides have emerged since 1980. In the recent times the research work for the production of fructooligosaccharides using microorganisms especially fungi.In the present work four organisms (*Aureobasidium pullullans, Aspergillus niger, Aspergillus sydowii, penicillium citrinum*) has been selected for the production of fructooligosaccharides.

A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

Moramatsu *et al.* (1988) works on a invention which relates to a method for production on an industrial scale of the branched Fructooligosacchrides indicated by the general structural formula below using microorganisms or an enzyme produced by microorganisms which belong to the genus *Aspergillus*, treating a sucrose solution containing at least 30% sucrose with the mycelia or the enzyme prepared from the mycelia of the microorganism *Aspergillus sydowi* at a reaction temperature in the range of 40°-60°C, subsequently removing the branched fructooligosacchrides produced, and separating said branched fructooligosacchrides from other compounds produced.

Han *et al.* (2002) works on a novel microorganism and a method for producing fructooligosaccharides and neofructooligosaccharides. More particularly, the work relates to *Penicillium citrinum* KCTC 10225BP of soil origin which produces

fructosyl transferase and hydrolyzes sucrose into fructooligosaccharides.

A fructose transferring enzyme is immobilized by adsorption on a granular carrier having a primary to quaternary amine. The carrier is preferably an epoxy polymer, a vinyl polymer or a chitosan derivative having a primary, secondary or tertiary amine. Immobilization can be performed without or with a cross-linking agent. The immobilized enzyme is used for producing fructooligosaccharides by passing a sucrose solution through a column containing the immobilized enzyme (Kono *et al.* 1992).

Park *et al.* (2002) works on a process for preparing beta-fructofuranosidase enzyme and a process for producing fructooligosaccharides, in which the preparation of the enzyme is obtained by cultivating the fungus *Aspergillus niger*, either wild or mutated, in a preferably semi-solid culture medium, in order to produce an extracellular enzyme, which is submitted to transfructosylation for producing fructooligosaccharides comprising sugars which are formed by one unit of sucrose and by two, three and four units of fructose.

PROPOSED METHODOLOGY OF THE PROPOSED WORK

Collection of culture of *Aureobasidium pullullans*

The pure culture of ***Aureobasidium pullullans*** for National Collection of Industrial Microorganisms (N.C.I.M.) PUNE cultures no N.C.I.M. No. 1049 and ATCC No. 9348. These organisms are responsible for production of FTase enzyme.

The maintain of culture of *Aureobasidium pullullans*

After collection of pure culture by N.C.I.M or ATCC reserved the culture of glysoral slants for further used. Kept in slant in -20^{o}C in Deep freezer or preserved in refrigerator at 4^{o}C for further use. Revised the culture after three months

MICROORGANISM USED

Aureobasidium pullullans

Taxonomic Classification;-

Kingdom	Fungi
Phylum	Ascomycota
Class	Euascomycetes
Order	Dothideales
Family	Dothioraceace
Genus	Aureobasidium
Species	Pullullans

- It is characterized as ambiguous saprophyte. The habitat of Aureobasidium pullullans of lies within the aerial parts plant because it is often the domination fungus found on leaves.

- It is also grows in the surface layers of many types of soil where it increases in abundance following nitrogen fertilization.

- This species has been isolated from fresh and salt water / sediments, the rhizosphere of grasses, seeds honeycombs, nests and feathers of living bird, frozen fruitcake, leather, cotton fabrics, concrete surfaces, paint, plastics optical lenses and human lymph nodes. Most strains grow optimally at 25 ^{o}C and–are–sensitive to heat although some isolates adapted to growth in lower and higher temperatures have been described.

- This species grows fairly rapidly, often maturing within 7 days of room temperature incubation on potato or glucose agar. Young colonies, composed primarily budding of yeast cells, are flat, smooth, and shiny. They range in colour from white to pink or yellow as the colonies mature. They develop a velvety texture and dark brown or black colour with a grayish fringe. At these stages hyphae projections and slimy masses of conidia appear.

- Hyphae are septate , up to 20 micro meter in diameter and initially appear hyaline but develop dark brown pigmentation with market intercalary constructions as they age Aureobasidium has no distinct conidiophores apical or intercalary blastoconidia are produced synchronously in tufts from undifferentiated hyphae.

Production of FOS through Immobilization

The production of FOS is primarily important to study different physical and chemical parameters, so the production of FOS firstly will be done by seed cultivation in which specific identified microbes will be allowed to grow sufficiently by providing different suitable cultivation conditions .The whole process will be done in 1000 ml conical flask containing 10 normal HCL and with 200 ml culture media. The seed culturing will be purely done in a shaker incubator with 150 rpm at a temperature of 28^{o}C for 40- 48 hours.

Cell immobilization and enzyme assay

- The Cell immobilization can be done by taking dry cells (20% w/v) from seed culture of a particular micro organism into a solution of sodium alginate at room temperature. The mixture will be extruded as small beads into 1% (w/v) of calcium chloride solution. The immobilized cells (hydrated beads) will be kept in tubes containing 100 ml of water as one tube will contain 30 beads respectively. The beaded tubes will be kept in defreeze at -15^{o}C for 24 hours to ensure freeze dehydration process. This will help to study enzyme kinetics by using different techniques (Tal *et al.* 1999).

- The enzyme activity will be determined by measuring the release of glucose into culture medium, it will be helpful to measure the turn over of a particular enzyme involved at different reaction conditions of temperature and pH.

- The whole enzyme assay will be done by following the procedure of (Yun *et al.* 2006) in which 1.5 ml sucrose will be prepared in 0.1M Sodium acetate buffer that will be added to 0.1 ml enzyme solution. After incubation period of 1 hour at 55^{o}C, 1 ml of dinitro salicylic acid will be added to terminate the reaction.

Cultural parameters

The followings physical and chemical parameters will be taken to observe the effects on the production of fructooligosacchrides.

1. Effect of temperature on enzyme activity during the production of FOS.

2. Effect of pH.

3. Effect of different carbon sources.

4. Effect of different incubation periods.

5. Effect of the process of agitation.

6. Effect of addition of amino acids and vitamins.

Analytical method of Fructooligosaccharides

The Fructooligosaccharides analyzed by high performance liquid chromatography (HPLC).

Chromatographic Conditions:-

Column: -	Shodex Asahipak NH_2 P-50 4E, 250 mm X4.6 mm X5.0 µ
Flow Rate: -	1.0ml / min
Detector: -	RI.
Injector Volume: -	10 µL.
RI Optical Unit Temperature: -	35 ^{0}C.
Column Temperature: -	30 ^{0}C.
Run Time: -	30 mins.
Reagent: -	Acetonitrile, Water

To 700 ml filtered acetonitrile, add 300 ml of filtered water, mix and degas.

Standard Preparation: - Weigh accurately about 10 mg each of Fructose, Glucose, and Sucrose, Kestose and Nystose, in 10 ml volumetric flask, add 5 ml of water and dissolve. Make up the volume to the mark with water.

Sample Preparation: - Weigh accurately a quantity of sample (about 1.0 g) into a 50 ml volumetric flask, add 30 ml of water and dissolve. Make up the volume to the mark with water. .

Procedure

- Wash the column initially with water. Acetonitrile (80:20) at a flow of 1ml /min for 30 minutes and then run mobile phase for 30 minutes.
- Inject blank (water) then the standard preparation (six injection) and sample preparation (two injection) and record the peak responses for fructose , glucose , sucrose, kestose, (GF) and nystose (GF3) . Calculate the content of fructose, glucose, sucrose, kestose (GF2) and nystose (GF3) of the FOS sample.

EXPECTED OUTCOME OF THE PROPOSED WORK

1. It may be helpful to screen out some particular micro organisms having potential to produce FOS.
2. It might be possible to detect particular range of a specific physical or chemical parameter which may have a crutial role to promote enzymatic activity for the enhanced production of FOS.
3. The production of fructooligosaccharides by the microorganism would be optimizing so as to get maximum production.

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Guide candidate